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Case Report

Fatal Adenovirus Infection Associated With New Genome Type

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The first fatal case caused by the new genome type 7i is described in an 8-month-old boy requiring long-term respiratory support who developed Reye's syndrome, acute respiratory distress, and bronchiolitis obliterans with fatal evolution. Adenovirus was detected in nasopharyngeal secretions and was persistently positive during hospitalization. IgM and IgG adenovirus antibody titers measured in serum by enzyme-linked immunoassay (EIA) were 1:32 and 1:800, respectively. Serum interleukins (IL) and interferons (IFN) measured by EIA were as follows: IL-2, 110 pg/ml; IL-6, 300 pg/ml; IL-8, 7,000 pg/ml; TNF-α, 35 pg/ml, IL-1 and IL-4 undetectable, IFN-α 2,200 pg/ml, and IFN-γ 700 pg/ml. Virologic studies showed that adenovirus isolated belonged to subgenus B, and digestion of viral DNA with Bam HI, Sma I, Bgl II, and Hind III identified the isolate as belonging to genome type 7i. Autopsy showed bronchiolitis obliterans with diffuse alveolar damage and perivenular fatty degeneration with polymorphonuclear infiltrates in the periportal spaces. The difficulty in obtaining adequate oxygenation with minimization of iatrogenic oxygen injury is discussed. ***J. Med. Virol. 54:233–236, 1998.***

KEY WORDS: adenovirus; acute respiratory infection; bronchiolitis obliterans; cytokines; interferons

INTRODUCTION

Among viruses involved in acute lower respiratory infection (ALRI), adenoviruses have been associated with severe and fatal disease as well as chronic airway obstruction. Adenovirus type 7 has been associated with epidemic outbreaks of disease, with considerable mortality, mainly in children [Nahmias et al., 1967]. Since the isolation of the prototype strain a total of 18 genome types designated 7a to 7j have been described and their geographic distribution reported [Li and Wadell, 1986; Niel et al., 1991; Kajon and Wadell, 1992; Kajon et al., 1996].

Molecular epidemiology studies of adenovirus associated with ALRI has shown that genome type 7h, identified for the first time in South America in 1984, represents the most frequent of all characterized genome variants circulating in this area in the last 10 years [Niel et al., 1991; Kajon and Wadell, 1992; Kajon et al., 1996]. Although adenovirus 7h is still the predominant virulent genome type of serotype 7 in the region, the recent occurrence of new DNA variants (7i and 7j) in association with ALRI shows a genetic shift of the virus [Kajon et al., 1996].

CASE REPORT

An 8-month-old boy was admitted to R. Gutiérrez Children's Hospital with fever and pneumonia. The child had been in good health up to 39 days before onset, when he was hospitalized with measles and discharged 3 days later. Three weeks later, he developed intermittent fever, otitis, and pneumonia, and was treated as an outpatient with amoxicillin, with prompt clinical improvement. Readmitted a fortnight later due to pneumonia recurrence, physical examination disclosed a pale, eutrophic (weight 7,850 g), afebrile, and lethargic child, with cervical and inguinal adenopathies. Chest X-ray showed bilateral reticular infiltrates in upper and lower left lung lobes. Laboratory data showed hematocrit 30% and white blood cell count

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17,000/mm^3 with 32% polymorphonuclear leukocytes and 62% lymphocytes. Arterial blood gas values at room air were pCO_2 40 mm Hg, $pO_2$98 mm Hg, oxygen saturation 97.8%, and pH 7.40. Although blood, urine, and CSF cultures for bacteria and fungi were negative, ampicillin and cefaclor were prescribed. Four days later the patient became febrile, hyperpneic, cyanotic, with hypoxemia, hypercapnia, and metabolic acidosis. Nasopharyngeal secretions were examined by immunofluorescence for adenovirus, respiratory syncytial virus, influenza A and B, and parainfluenza 1, 2, and 3 [Bulletin World Health Organization, 1977]. Upon detection of adenovirus, secretions were inoculated onto HEp-2 and A 549 cells; adenovirus was isolated and characterized by restriction enzyme analysis [Kajon et al., 1996; Kajon and Wadell, 1994]. The patient was treated empirically with tuberculostatic drugs. Generalized tonic-clonic seizures developed and mechanical ventilation was started due to excessive respiratory workload. The liver was 7 cm below the right costal margin and the spleen was 2.5 cm below. Serum alanine was 384 IU/L, aspartate-aminotransferase 2,840 IU/L, ammonia 103 μmol/L, and prothrombin time was 20%. IgMs test for hepatitis A, B, and Epstein-Barr viruses were negative. Both Mantoux test (PPD = 2 TU) and antibodies against *Mycobacterium tuberculosis,* measured by enzyme-linked immunoassay (EIA) were negative as well as *Mycobacteria* detection in bronchoalveolar lavage, urine, and CSF. Cerebral echography showed vasculitis without meningeal compromise and CSF analysis was normal. Reye's syndrome associated with adenoviral infection was suspected. Tuberculosis was thought unlikely and its treatment suspended. Three days later an hepatogram showed a decrease in transaminases and improvement in liver function. The patient developed a progressive and severe hypoxemia and acute respiratory distress syndrome (ARDS). Adenovirus detection in nasopharyngeal secretions remained positive. Serial dilution of patient's serum (between 1:32 and 1:4,096) was done to measure IgM and IgG anti-adenovirus titers by EIA [Mistchenko et al., 1992]. The test was considered positive when the absorbance value of the sample was greater than three-fold the negative control value; titers obtained were 1:32 and 1:800 for IgM and IgG, respectively. Due to the limited volume of serum available, the presence of neutralizing antibodies against adenovirus 7 could not be assessed. Circulating immune complexes containing adenovirus hexon were found in serum samples by EIA [Mistchenko et al., 1992] and complement fractions C3 and C4 were decreased. White blood cell count increased to 34,400/mm^3 with 30% polymorphonuclear cells. Serum cytokines measured by EIA (R&D Systems, Minneapolis, MN) were as follows: IL-2, 110 pg/ml; IL-6, 300 pg/ml; IL-8, 7,000 pg/ml; TNF-α, 35 pg/ml; IL-1 and IL-4 undetectable. Interferon values measured by EIA (Endogen Inc., Woburn, MA) were IFN-α, 2,200 pg/ml, and IFN-γ 700 pg/ml. Mechanical ventilation was continued with FiO_2 between 0.6 and 1 and oxygen saturation lower than 90%; he developed pneumomediastinum and left pneumothorax. Fifteen days later, the patient showed signs of pulmonary hypertension, emphysema, and cyanosis. Despite treatment with antibiotics, inotropic vasoactive agents, cardiovascular and respiratory functions deteriorated, and the child died in respiratory failure 32 days after admission.

Partial autopsy (excluding brain, due to parents' refusal) disclosed bronchiolitis obliterans with diffuse alveolar damage. Bronchi and bronchioles showed epithelial hyperplasia, while alveolar sacs and ducts displayed proliferation of type II pneumonocytes with thickening and fibrosis of interalveolar septa. Lymphocytic infiltrate was scarce and located in bronchial walls and interalveolar septa. Rare smudge cells were observed. The liver showed perivenular fatty degeneration with polymorphonuclear infiltrates in the periportal space.

Virologic studies based on digestion of viral DNA with Bam HI and Sma I demonstrated that adenovirus isolated belonged to subgenus B. Additional analysis with restriction endonucleases Bam HI, Sma I Cfo I, Bgl II, and Hind III identified the isolate as belonging to genome type 7. Figure 1 shows the restriction profile obtained with Bam HI and Cfo I which allowed identification of the isolate as genome type 7i [Kajon et al., 1996].

DISCUSSION

A case is described of a child who developed bronchiolitis obliterans with fatal outcome, associated with a new genome type of adenovirus, designated as 7i. The patient had a post-measles adenovirus infection which evolved slowly and progressively to culminate in complete lung function deterioration. Measles infection is associated with a significant depression in the total number of circulating T cells, particularly the $CD4^+$ subset, so that transient immunosuppression increases the risk of developing severe secondary infections, mainly by adenovirus [Sly et al., 1984; Griffin and Ward, 1993].

In our patient, early recognition of bronchiolitis obliterans and prompt initiation of corticosteroid therapy could have improved survival. The acute onset of generalized seizures, the marked increase in serum aminotransferase level, and prothrombin time in an infant infected with adenovirus suggested Reye's syndrome. Liver dysfunction with fatty degeneration of hepatocytes was also present, in the absence of cerebral edema, hypoglycemia, and hyperammoniemia [Edwards et al., 1985]. These findings fail to meet the diagnostic criteria of Reye's syndrome, but are consistent with the pseudo-Reye's syndrome described by Ladisch et al. [1979] in adenoviral infection.

The patient then developed distributive shock and ARDS, following a striking increase in peripheral blood leukocytes. The role of leukocytes and cytokines in damage to endothelial and epithelial lung surfaces in ARDS has been widely reported [Meduri et al., 1995]. In our patient, high serum levels of pro-inflammatory

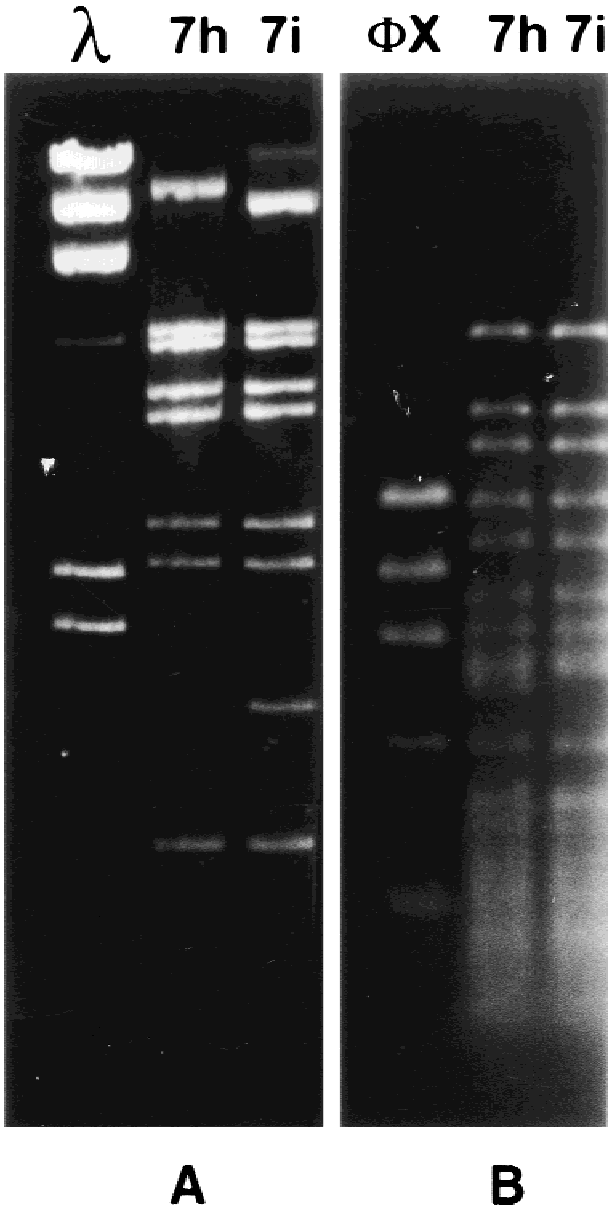

Fig. 1. Adenovirus genome type 7i and closely related 7h digested with *Bam*HI **(A)** and *Cfo* I **(B).** Molecular weight markers: λ DNA digested with *Hin*d III and φX DNA digested with *Hae* III.

cytokines IL-6, IL-8, and TNF-α were detected at the onset of ARDS, in agreement with the high levels of these cytokines previously described in association with severe adenovirus infection in children [Mistchenko et al., 1994]. TNF-α causes ARDS-like lung injury in vivo by decreased expression of surfactant protein B.

Airway inflammatory responses result from complex interaction between cytokines, chemoattractants, and cell adhesion molecules. Intercellular adhesion molecule-1 (ICAM-1) is expressed constitutively on endothelium and its level of expression is increased by some cytokines. Adenoviruses increase ICAM-1 expression on airway epithelial cells which could promote the development of airway inflammation [Pilewski et al., 1995].

Potential mechanisms for the development of an inflammatory response against adenovirus infection have been reported, including: (i) tissue deposit of immune complexes containing adenovirus antigen; (ii) production of proinflammatory cytokines IL-6, IL-8, and TNF-α; (iii) increased expression of ICAM-1 in the respiratory airway, and (iv) increased granulocyte adherence to endothelium.

Human adenovirus pneumonia may present either an acute or chronic histopathological pattern. In addition to macrophage infiltrate, in acute infection type II pneumonocytes with inclusion bodies are readily found. In contrast, in chronic infection adenovirus-infected cells are scarce, the infiltrate is lymphocytic and interstitial proliferation of fibroblast-like cells can be found, as well as an increase in collagen types I and III and elastic fibers [Rosman et al., 1996]. Persistent adenovirus infection may enhance lung damage.

In our patient, in addition to viral lung damage, secondary lung injury caused by long-term mechanical ventilation should be considered. Toxic reactions to oxygen occur commonly with the use of FiO_2 greater than 0.5 for over 24 hours. Hyperoxia produces progressive changes in alveolar interstitium, involving both cells and extracellular matrix, as well as polymorphonuclear neutrophil infiltration. Lastly, hyperoxia stimulates human alveolar macrophages to release biologically active IL-8 and alters surfactant phospholipid metabolism [Crim and Longmore, 1995]. It is to be hoped that improved treatment may avoid chronic lung damage secondary to aggressive therapy liable to affect survival.

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